BIOPRESERVATION, AN ECOLOGICAL APPROACH TO IMPROVE THE SAFETY AND SHELF-LIFE OF FOODS

Dr. Razzagh Mahmoudi (DV.M. phD)

Introduction

- Modern technologies implemented in food processing and microbiological food-safety standards have diminished, but not altogether eliminated, the likelihood of food-related illness and product spoilage in industrialized countries.

- The increasing consumption of precooked food, prone to temperature abuse, and the importation of raw foods from developing countries are among the main causes of this situation.

Introduction

- Hence, in Europe, morbidity from foodborne llnesses is second only to respiratory diseases, with estimates of 50,000 to 300,000 cases of acute gastroenteritis per million population every year.

- In the USA, acute gastroenteritis affects 250 to 350 million people annually, and an estimated 22% to 30% of these cases are thought to be foodborne diseases with the main foods implicated including meat, poultry, eggs, seafood, and dairy products

Introduction

- The bacterial pathogens that account for many of these cases include *Salmonella, Campylobacter jejuni, Escherichia coli 0157:H7, Listeria monocytogenes, Staphylococcus aureus, and Clostridium botulinum.*

- *Until now, approaches to seek improved food safety have relied on the search* for more efficient chemical preservatives or on the application of more drastic physical treatments (e.g. high temperatures).

Introduction

- Nevertheless, these types of solutions have many drawbacks: the proven toxicity of many of the commonest chemical preservatives (e.g. nitrites), the alteration of the organoleptic and nutritional properties of foods, and especially recent consumer trends in purchasing and consumption, with demands for safe but minimally processed products without additives.

Introduction

- To harmonize consumer demands with the necessary safety standards, traditional means of controlling microbial spoilage and safety hazards in foods are being replaced by combinations of innovative technologies that include biological antimicrobial systems such as lactic acid bacteria (LAB) and/or their bacteriocins.
- The use of LAB and/or their bacteriocins, either alone or in combination with mild physicochemical treatments and low concentrations of traditional and natural chemical preservatives, may be an efficient way of extending shelf life and food safety through the inhibition of spoilage and pathogenic bacteria without altering the nutritional quality of raw materials and food products.

Biological methods for food preservation

- Biopreservation, as commented above, can be defined as the extension of shelf life and food safety by the use of natural or controlled microbiota and/or their antimicrobial compounds.

- One of the most common forms of food biopreservation is fermentation, a process based on the growth of microorganisms in foods, whether natural or added.

Biological methods for food preservation

- These organisms mainly comprise lactic acid bacteria, which produce organic acids and other compounds that, in addition to antimicrobial properties, also confer unique flavours and textures to food products.

- Traditionally, a great number of foods have been protected against spoiling by natural processes of fermentation.

- Currently, fermented foods are increasing in popularity (60% of the diet in industrialized countries).

Biological methods for food preservation

- to assure the homogeneity, quality, and safety of products, they are produced by the intentional application in raw foods of different microbial systems (starter/protective cultures).

- The starter cultures of fermented foods can be defined as preparations of one or several systems of microorganisms that are applied to initiate the process of fermentation during food manufacture, fundamentally in the dairy industry and, currently, extended to other fermented foods such as meat, spirits, vegetable products, and juices.

Biological methods for food preservation

- The bacteria used are selected depending on food type with the aim of positively affecting the physical, chemical, and biological composition of foods, providing attractive flavour properties for the consumer.

- To be used as starter cultures, microorganisms must fulfil the standards of GRAS status (Generally Recognized As Safe by people and the scientific community) and present no pathogenic nor toxigenic potential.

Lactic Acid Bacteria

- LAB include the genera *Lactococcus, Streptococcus, Lactobacillus, Pediococcus, Leuconostoc, Enterococcus, Carnobacterium, Aerococcus, Oenococcus, Tetragenococcus, Vagococcus, and Weisella*.

- They form a natural group of Gram-positive, nonmotile, non-sporeforming, rod- and coccus-shaped organisms that can ferment carbohydrates to form chiefly lactic acid; they also have low proportions of G+C in their DNA (< 55%).

Lactic Acid Bacteria

- LAB present attractive physiological properties and technological applications (resistance to bacteriophages, proteolytic activity, lactose and citrate fermentation, production of polysaccharides, high resistance to freezing and lyophilization, capacity for adhesion and colonization of the digestive mucosa, and production of antimicrobial substances).

- In general, LAB have GRAS status and play an essential role in food fermentation given that a wide variety of strains are employed as starter cultures (or protective cultures) in the manufacture of dairy, meat, and vegetable products.

Lactic Acid Bacteria

- The most important contribution of these microorganisms is the preservation of the nutritional qualities of the raw material through extended shelf life and the inhibition of spoilage and pathogenic bacteria.
- This contribution is due to:
  - competition for nutrients
  - presence of inhibitor agents produced, including organic acids, hydrogen peroxide, and bacteriocins.

Lactic Acid Bacteria

- There are many reviews on reported examples of spoilage and pathogenic bacteria inhibition by bacteriocin-producing LAB.
- In addition to the food applications of LAB, various strains are considered to be probiotics.
- Probiotics can be described as a preparation of or a product containing viable, defined microorganisms in sufficient numbers to alter the microbiota (by implantation or colonization) in a compartment of the host and that exert beneficial health effects in this host.

Lactic Acid Bacteria

- In this regard, LAB fit many of requirements for a icroorganism to be defined as an effective probiotic.

- These requirements include the ability to:

(a) adhere to cells

(b) exclude or reduce pathogenic adherence;

(c) persist and multiply

(d) produce acids, hydrogen peroxide and acteriocins antagonistic to pathogen growth

(e) be safe, noninvasive, noncarcinogenic and nonpathogenic

(f) coaggregate to form a normal balanced flora

LAB bacteriocins

- The antimicrobial ribosomally synthesized peptides produced by bacteria, including members of the LAB, are called bacteriocins.

- Such peptides are produced by many, if not all, bacterial species and kill closely related microorganisms.

- Due to their nature, they are inactivated by proteases in the gastrointestinal tract.

LAB bacteriocins

- Most of the LAB bacteriocins identified so far are thermostable cationic molecules that have up to 60 amino acid residues and hydrophobic patches.

- Electrostatic interactions with negatively charged phosphate groups on target cell membranes are thought to contribute to the initial binding, forming pores and killing the cells after causing lethal damage and autolysin activation to digest the cellular wall.

LAB bacteriocins

- **Example of damage caused by bacteriocin** on *L. monocytogenes CECT 4032 cells.*
- ***(A) cells*** without enterocin AS-48
- **(B) cells treated with** 0.1 µg/ml of AS-48 for 2 h
- **(C and D) cells** treated with 3 µg/ml of enterocin AS-48 for 10 min (adapted from.

LAB bacteriocins

- The LAB bacteriocins have many attractive characteristics that make them suitable candidates for use as food preservatives, such as:

- Protein nature, inactivation by proteolytic enzymes of gastrointestinal tract
- Non-toxic to laboratory animals tested and enerally non-immunogenic
- Inactive against eukaryotic cells
- Generally thermoresistant (can maintain antimicrobial activity after pasteurization and sterilization)

LAB bacteriocins

- Broad bactericidal activity affecting most of the Gram-positive bacteria and some, damaged, Gram-negative bacteria including various pathogens such as *L. monocytogenes, Bacillus cereus, S. aureus, and Salmonella*

- Genetic determinants generally located in plasmid, which facilitates genetic manipulation to increase the variety of natural peptide analogues with desirable characteristics.

LAB bacteriocins

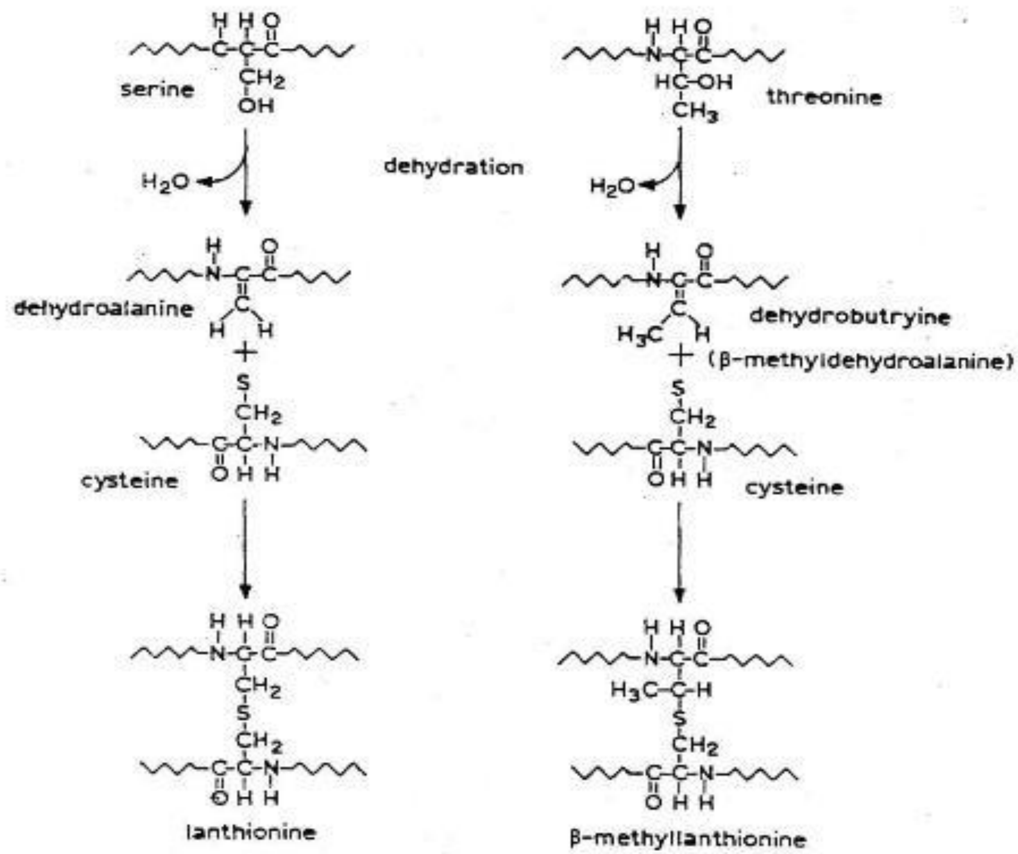
- For these reasons, the use of bacteriocins has, in recent years, attracted considerable interest for use as biopreservatives in food, which has led to the discovery of an ever-increasing potential of these peptides.
- Undoubtedly, the most extensively studied bacteriocin is nisin, which has gained widespread applications in the food industry.
- This FDA-approved bacteriocin is produced by the GRAS microorganism *Lactococcus lactis and is used as a food additive in at least 48 countries, particularly in* processed cheese, dairy products and canned foods.
- Nisin is effective against food-borne pathogens such as *L. monocytogenes and many other Gram-positive spoilage microorganisms*

Classification of LAB bacteriocins

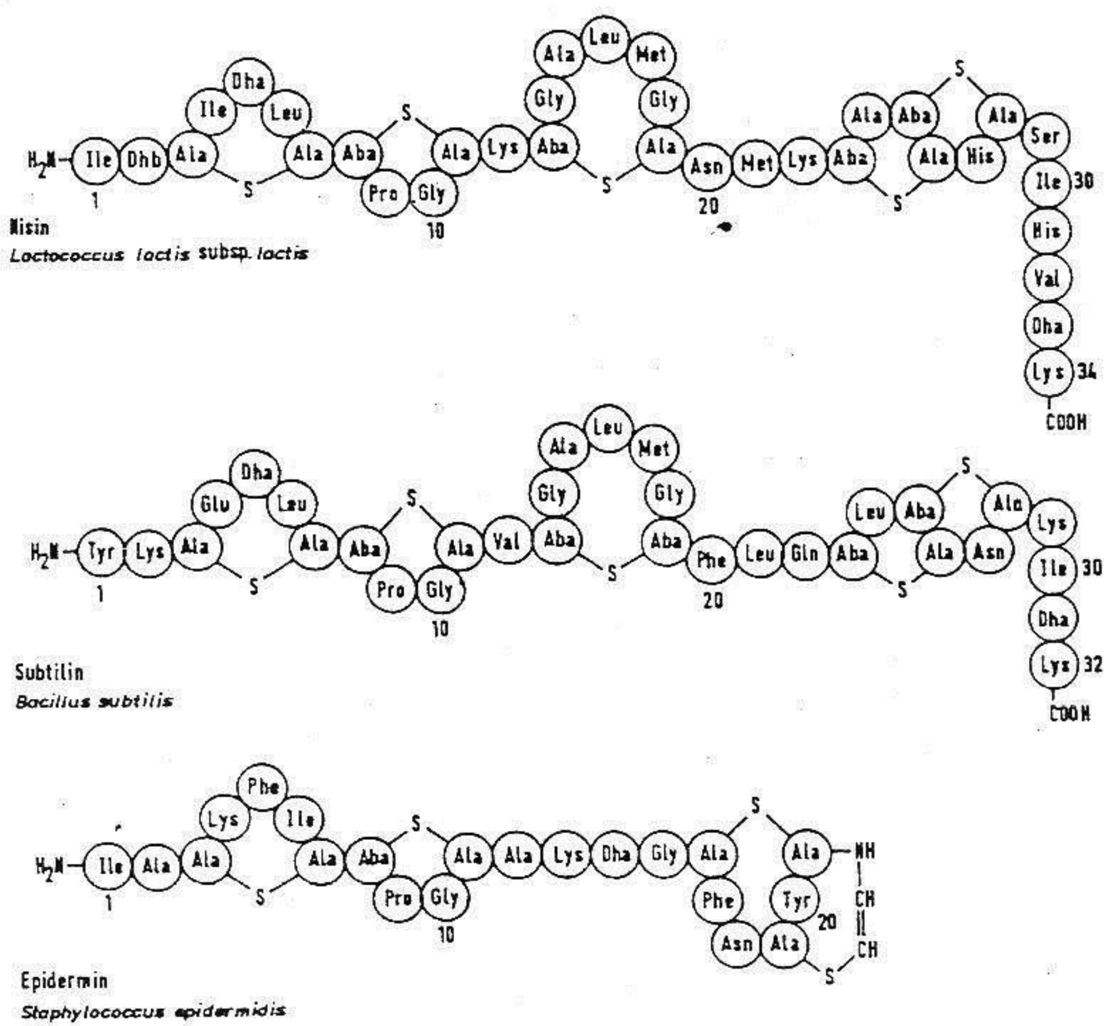
- LAB bacteriocins were divided into four classes, with class II further divided into three subclasses. However, class IV was later eliminated and bacteriocins in class II were regrouped by different authors. Recently, [38] have proposed four classes for Gram- positive bacteriocins that could also be applied to LAB bacteriocins.

Classification of LAB bacteriocins

- *Class I comprises the lantibiotics (lanthionine- containing peptides with antibiotic activity).*
- *They are* small peptides that are differentiated from other bacteriocins by their content in dehydroamino acids and thioether amino acids.
- include nisin, discovered in 1928, lacticin 481 of *L. lactis, citolysin* of *E. faecalis, and lacticin 3147* of *L. lactis, among* others.

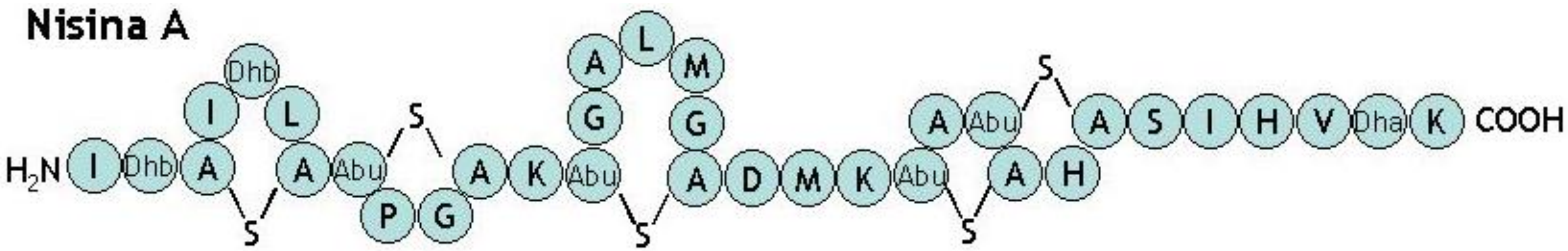


serine
threonine
dehydration
H2O
dehydroalanine
dehydrobutryine
(β-methyldehydroalanine)
cysteine
lanthionine
β-methyllanthionine



Structure of nisin and related lantibiotics. Dha, dehydroalanine; Dhb, dehydrobutyrine; Ala-S-Ala, lanthionine; Aba, α-aminobutyric acid; Aba-S-Ala, β-methyllanthionine. All α-carbon atoms of lanthionine and β-methyllanthionine are in the D-configuration. Adapted from Gross and Morell, 1971; Gross, 1975; Schnell *et al.*, 1988.

Structure of nisin A.



Classification of LAB bacteriocins

- *Class II comprises the (<10 kDa) thermostable non-lantibiotic linear peptides.*
- *They are divided into* three subclasses on the basis of either a distinctive N-terminal sequence, the pediocin-like bacteriocins (class II.1), the lack of leader peptide (class II.2), or neither of the above traits (class II.3).
- Examples of the three subclasses are pediocin PA-1/AcH produced by *Pediococcus*, *enterocin EJ97 by E. faecalis*, and enterocin L50A by *E. faecalis , respectively.*

Classification of LAB bacteriocins

- *Class III includes the large (> 30 KDa) heat-labile bacteriocins that encompass many bacteriolytic* extracellular enzymes (hemolysins and uramidases) that may mimic the physiological activities of bacteriocins.
- Examples are helveticin J of *L. helveticus*
- *bacteriocin Bc-48 of E. faecalis.*

Classification of LAB bacteriocins

- *Class IV is a new class created to include the circular antibacterial peptide, an intriguing and novel type of* antimicrobial substance produced not only by bacteria but also by plants and mammalian cells.
- The distinguishing characteristic is the existence of head-to-tail peptide chain ligation, which makes them molecules with neither an origin nor an end.
- The first circular protein described was the enterocin AS-48 (reviewed in [46]).

Classification of LAB bacteriocins

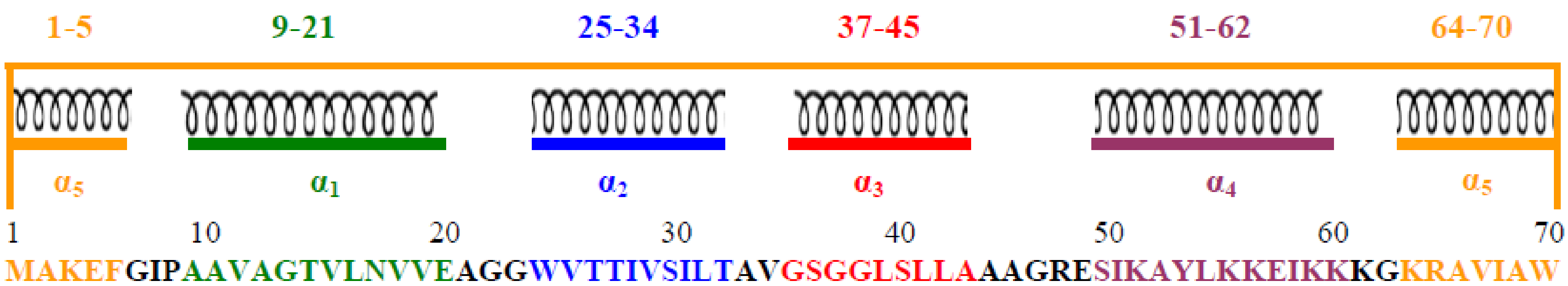


Fig. 3 Representation of primary (below) and secondary (above) structures of enterocin AS-48 with the head-to-tail ligation shown.

Effectiveness of bacteriocins in food systems

- The application of bacteriocins, particularly nisin, in food systems has been extensively reviewed. It is now known that the production and activity of bacteriocins in foods can be influenced by many factors

• Factors negatively affecting production include:

inadequate physical conditions and chemical composition of food (pH, temperature, nutrients, etc.)

spontaneous loss in production capacity

inactivation by phage of the producing strain

antagonism effect of other microorganisms in foods.

Nisin, for example, is 228 times more soluble at pH 2 than at pH 8.

Effectiveness of bacteriocins in food systems

- The effectiveness of bacteriocin activity in food is negatively affected by:
- Resistance development of pathogens to the bacteriocin
- Inadequate environmental conditions for the biological activity
- Higher retention of the bacteriocin molecules by food system components (e.g. fat)
- Inactivation by other additives
- Slower diffusion and solubility and/or irregular distribution of bacteriocin molecules in the meat matrix [50, 53].

Requirements and regulatory status for bacteriocins

- In general, the following features should be considered when selecting bacteriocin-producing strains for food applications:

- The producing strain should preferably have GRAS status.
- Depending on the application, the bacteriocin should have a broad spectrum of inhibition that includes pathogens or else high specific activity.
- Thermostability.
- Beneficial effects and improved safety.
- No adverse effect on quality and flavour.

Applications of bacteriocin-producing LAB in food

- The strategies for the application of LAB and/or bacteriocins in food are diverse:

• Inoculation of food with LAB (starter cultures or protective cultures) where bacteriocins are produced *in situ*

• Use of food previously fermented with the bacteriocin-producing strains as an ingredient in the food processing

• Addition of purified or semipurified bacteriocins. The purified bacteriocins are considered additives and always require express authorization for their use.

Application of bacteriocins in dairy products

- Several researchers have demonstrated the effectiveness of nisin and/or nisin-producing strains against pathogenic bacteria such as *Clostridium butulinum in cheese and against L. monocytogenes in* cheeses such as Camembert, Ricotta, Feta and Manchego.
- Other bacteriocins have been tested in milk and dairy products, such as pediocin AcH in milk and Cheddar and Munster cheeses against *L. monocytogenes, S. aureus, and E. coli O157:H7*, lacticin 3147 against undesirable LAB, *L. monocytogenes and B. cereus in Cheddar, Cottage cheese and* yogurt, and enterocin AS-48 against *B. cereus, S. aureus and L. monocytogenes in milk and* Manchego cheese.

Applications in meat products

- When evaluating a bacteriocin-producing culture for sausage fermentation and/or biopreservation, one must bear in mind that meat and meat products are complex systems with a number of factors influencing microbial growth and metabolite production.
- Therefore, the influence of formula and fermentation technology on the performance of bacteriocin-producing cultures needs to be assayed.

Applications in meat products

- The most-studied bacteriocins in meat and meat products include nisin, enterocin AS-48, enterocins A and B, sakacin, leucocin A, and especially pediocin PA-l/AcH, alone or in combination with several physicochemical treatments, modified atmosphere packaging, high hydrostatic pressure, (HHP), heat, and chemical preservatives, as an additional hurdle to control the proliferation of *L. monocytogenes and* other pathogens.

- Furthermore, several bacteriocinogenic LAB have been used as bioprotective cultures for food manufacturing processes in attempts to control these pathogens.

Applications in meat products

- The data available on the use of nisin in cured and fermented meat are equivocal.
- Compared to dairy products, nisin use in meat products has not been very successful because of its low solubility, irregular distribution, and lack of stability.
- Pediocin PA-I/AcH is more suitable for use in meat and meat products than nisin
- however, *P. acidilactici is not an indigenous meat strain.*

Applications in vegetable products

- Tests of bacteriocins in vegetable products include nisin in tinned vegetables and fruit juices.
- pediocin PA-1/AcH in salad and fruit juice
- enterocin AS-48 against *B. cereus in rice* and vegetables and in fruit juices against other pathogens such as *E. coli O157:H7*, *S. aureus,* and the spoilage bacterium *Alicyclobacillus acidoterrestris.*

Applications in fish

- The deterioration of fresh fish is generally caused by Gram-negative microorganisms; however, in vacuum-packed fresh fish and seafood, pathogenic organisms such as *Clostridium botulinum and L. monocytogenes can also cause problems.*

- Scant work has focused on incorporating live bacteriocinproducing cultures into these products or on the addition of concentrated bacteriocin preparations.

Applications in fish

- The combination of nisin and Microgard reduced the total aerobic bacteria populations of fresh chilled salmon, increased its shelf-life, and also reduced the growth of innoculated *L. monocytogenes in frozenthawed* salmon.

- The inhibition of *L. monocytogenes was also confirmed with other bacteriocinproducer* cultures such as *Carnobacterium divergens.*

- demonstrated the synergistic effect of combination lactic acid, sodium chloride, and/or nisin in rainbow trout, and more recently showed the effect of LAB cultures on pathogenic microorganism control in fish.

Hurdle technology for food preservation

- The hurdle concept was introduced by Leistner in 1978 and stated that the microbial safety, stability, sensorial, and nutritional qualities of foods are based on the application of combined preservative factors (called hurdles) that microorganisms present in the food are unable to overcome.

- Thus, hurdle technology refers to the combination of different preservation methods and processes to inhibit microbial growth.

Hurdle technology for food preservation

- The principal hurdles employed in food safety are:

temperature (higher or lower)

aw

pH

Eh

Chemical preservatives

vacuum packaging

modified atmosphere

HHP

UV

competitive flora (LAB producing antimicrobial compounds)

Applications of LAB bacteriocins in hurdle technology

- Several authors have recommended the use of bacteriocins combined with other preservation methods to create a series of hurdles during the manufacturing process to reduce food spoilage by microorganisms.

- In fact, it has been proven that the application of chemical preservatives, physical treatments (heat), or new mild non-thermal physical methods (pulsed electric field, HHP, vacuum, or modified atmosphere packaging), which increase the permeability of cell membranes, positively affects the activity of many bacteriocins.

Applications of LAB bacteriocins in hurdle technology

- Notably, combined treatments of bacteriocins with selected hurdles affecting outer-membrane (OM) permeability increase the effectiveness of some LAB bacteriocins against Gram-negative cells, which are generally resistant.

- Concretely, the growth of Gramnegative pathogens such as *E. coli O157:H7 and Salmonella can also be controlled when metal chelators,* such as EDTA, sodium tripolyphosphate (STPP) or physical methods such as heat and HHP, are used in combination with bacteriocins.

-

Table 1 Examples of bacteriocins used as a part of hurdle technology to control pathogen and spoiling microorganisms in foods.

Bacteriocin	Other hurdles	Results	Reference
Nisin	HHP	Combination of HHP and nisin was effective to inactivate cheese indigenous microbiota. This combination was also effective against *S. carnosus* and *B. subtilis* spores, although a part of population survived the treatment	[101]
	pH and low temperature	A significant reduction in *L. innocua* was observed with a combination of low pH 5.5 and nisin at 20 °C. However, nisin-resistant cells regrew. Additional hurdles, such as refrigeration temperature, caused a dramatic reduction in population and allowed an increase of storage time to 10 days in liquid cheese whey.	[102]
	Pulsed electric fields (PEF)	The addition of nisin prior to PEF treatment increased the susceptibility of *L. innocua* to PEF treatment in whey.	[103]
	Sodium citrate and sodium lactate	The combination of low temperature, sodium lactate and/or sodium citrate with nisin controls *Arcobacter butzleri* on chicken.	[104]
Pediocin AcH	HHP and high temperature	The combination of HHP , higher temperature, and pediocin acts synergistically, causing reduction of viability of *S. aureus*, *L. monocytogenes*, *E. coli* O157:H7, *Lb. sakei*, *Le. mesenteroides*	[105]
	Sodium diacetate	Combination of pediocin and sodium diacetate works synergistically against *L. monocytogenes* at room and low temperature	[106]
Enterocins A and B	HHP	Enterocins A and B were used in combination with HHP to the enhancement of safety in cooked ham against *L. monocytogenes*. Pathogen counts were below detection limits at the end of storage.	[72]
Enterocin AS-48	Heat treatment	The efficacy of AS-48 against *S. aureus* was greatly enhanced by combination with a moderate heat treatment in milk.	[68]
	STPP, lactic, acetic and citric acids	The combination of AS-48 and STPP or lactate acts synergistically against *S. aureus*. The activity of AS-48 increases in the presence of organic acids at pH 4.5. The combination with lactate reduces *S. aureus* population by 6 log units under neutral pH.	[107]
	Mild heat treatment, OM-permeabilizing agents or acidic/alkaline pH	The antimicrobial activity of AS-48 against *E. coli* O157:H7 enhanced by combination with mild heat treatment, OM-permeabilizing agents (EDTA and STPP), or under acidic or alkaline conditions in buffer and in apple juice.	[85]
	NaCl and low temperature	Highest effectiveness of AS-48 against *S. aureus* was obtained at 4 °C in combination with high concentrations of NaCl (6 and 7%).	[100]

TABLE 7.3
International Regulations on Nisin Use

Geographic Area	Food in Which Nisin Is Permitted	Maximum Addition Level (µg/g)
Abu Dhabi	Pasteurized, flavored, and long-life milks; processed cheese; other dairy products; canned foods	No limit
Algeria	Processed cheese	2.5
Argentina	Cheese, processed cheese, requeijao, and ricotta	12.5
Australia[a]	Crumpets, flapjacks, and pikelets (hot plate products)	25
	Cheese, processed cheese, and reduced-fat processed cheese and cheese spreads; processed cheese food; club cheese; blended cheese; canned tomatoes; tomato paste and tomato puree with pH <4.5; canned soups given a botulinum process; beer	No limit
Bahrain	Pasteurized, flavored, and long-life milks; processed cheese; cheese; other dairy products; canned foods	No limit
Bolivia	Permitted additive	No limit
Brazil	Cheese, processed cheese, pasteurized cheese, requeijao, and ricotta	12.5
Bulgaria	Cheese	5
	Ice for storing fish	No limit
Chile	Cheese	12.5
China	Canned foods and plant protein	5
	Dairy and meat produce	12.5
Colombia	Cheese	12.5
Costa Rica	Cheese products	No limt
Croatia	Cheese	12.5
	Mascarpone	10
Cyprus	Cheese, clotted cream, canned vegetables	No limit
Czech Republic	Cheeses	12.5
	Semolina, tapioca and similar puddings	3
Ecuador	All foods	Not specified
Egypt	Processed cheese and processed cheese paste	12.5
Estonia	Clotted cream	10
	Ripened and processed cheese	12.5
	Semolina, tapioca and similar puddings	3
European Union	Clotted cream	10
	Ripened and processed cheese	12.5
	Semolina, tapioca and similar puddings	3
	Mascarpone	10
Gibraltar	Canned foods (pH <4.5 or given botulinum process), cheese, clotted cream	No limit
Guyana	Canned foods, including canned meat, with pH <4.5, or given botulinum process, clotted cream	No limit
Hong Kong	Canned foods, cheese, clotted cream	No limit
Iceland	Ripened and processed cheese	12.5
	Semolina, tapioca and similar puddings	3
India	Processed cheese and cheese	100
	Coconut water	125
Indonesia	Cheese preparations	12.5
Israel	Cheese (except soft white cheese)	No limit
Jordan	Processed cheese and spreadable processed cheese	12.5

(continued)

International Regulations on Nisin Use

Geographic Area	Food in Which Nisin Is Permitted	Maximum Addition Level (μg/g)
Kuwait	Processed cheese and processed cheese preparations	12.5
Latvia	Clotted cream	10
	Ripened and processed cheese	12.5
	Semolina, tapioca and similar puddings	3
Lithuania	Clotted cream	10
	Ripened and processed cheese	12.5
	Semolina, tapioca and similar puddings	3
Macedonia	Processed cheese	12.5
Malaysia	Cheese, canned foods given a botulinum process	No limit
Malta	Cheese, clotted cream, canned foods	No limit
Mauritius	Canned foods, cheese, clotted cream	No limit
Mexico	Permitted additive	No limit
	Processed cheese	12.5
Montenegro	Processed cheese	12.5
Morocco	Clotted cream	10
	Ripened and processed cheese	12.5
	Semolina, tapioca and similar puddings	3
New Zealand	Processed cheese and cheese food, spreadable processed cheese, and processed cheese spread	12.5
	Beer	5
Panama	Fresh cheeses	No limit
Papua New Guinea	Tomato puree, canned tomato pulp, juice and paste, canned fruit (all with pH <4.5), cheese	No limit
Paraguay	Cheese, processed cheese, requeijao, ricotta	10
Peru	Permitted additive	No limit
Philippines	Processed cheese	100
Poland	Processed cheese	12
Qatar	Milk and milk products	No limit
Romania	Any food	2.5
Russia	Processed cheese	5
	Canned vegetables	2.5
Saudi Arabia	Processed cheese and processed cheese spread, processed cheese with vegetable oils, other foods and dairy products	12.5 No limit
Serbia	Processed cheese	12.5
Singapore	Cheese, canned foods given a botulinum process	No limit
Slovak Republic	Bakery products, sterilized and soused vegetables, concentrated milk products, desserts, cheese, ready meals, semi-canned products, mayonnaise and its products, sauces, creams, beer	12.5
Slovenia	Clotted cream	10
	Ripened and processed cheese	12.5
	Semolina, tapioca and similar puddings	3
	Mascarpone	10
South Africa	Cheeses, processed or blended cheese including cheese spread, processed cheese preparations, and soft cheese	12.5
Sri Lanka	Permitted additive	No limit
Taiwan	Cheese	125

(continued)

Typical Addition Levels of Nisin and the Commercial Extract Nisaplin

Food	Nisin (µg/g)	Nisaplin (mg/kg, mg/L)	Typical Target Organism
Processed cheese	2.5–15	100–600	*Bacillus, Clostridium*
Milk and milk products	0.25–1.25	10–50	*Bacillus (B. sporothermodurans)*
Pasteurized chilled dairy desserts	1.88–5.0	75–200	*Bacillus, Clostridium*
Liquid egg	1.25–5	5–200	*Bacillus (B. cereus)*
Pasteurized soups	2.5–6.25	100–250	*Bacillus*
Crumpets	3.75–6.25	150–250	*B. cereus*
Fruit juice (pasteurized/ambient storage)	0.75–1.5	30–60	*Alicyclobacillus acidoterrestris*
Canned food	2.5–5	100–200	*B. stearothermophilus, Cl. thermosaccharolyticum, Cl. botulinum*
Dressings and sauces	1.25–5	50–200	Lactic acid bacteria, *Clostridium, Bacillus*
Meat products such as bologna, frankfurter sausages	5–10	200–400	Lactic acid bacteria *Br. thermosphacta* *L. monocytogenes*
Ricotta cheese	2.5–5	100–200	*L. monocytogenes*
Beer, wine, fermented beverages, spirits			Lactic acid bacteria (*Lactobacillus, Pediococcus*)
Pitching yeast wash	25–37.5	1000–1500	
Reduced pasteurization	0.25–1.25	10–50	
During fermentation	0.63–2.5	25–100	
Postfermentation	0.25–1.25	10–50	

Source: Updated from Thomas et al., 2000.